



Effect of Crude oil contaminated water on the guinea pig (*Cavia porcellus*): Ileum morphological cum histological perspectives.

¹Mbadugha C. Christopher , ¹Aquisua N. Aquaisua, ¹Eno-obong I. Bassey,¹Aniekan I. Peter, ¹Edagha A. Innocent, ²Bankole J. Leko.

¹Department of Anatomy, Faculty of Basic Medical Sciences, University of Uyo, P.M.B.1017, Uyo, Akwa Ibom State, Nigeria.

²Department of Anatomy, Faculty of Basic Medical Sciences, Madonna University, Elele, Rivers State, Nigeria.

Corresponding author email: christophermbadugha@rocketmail.com

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ABSTRACT

Objective: is to determine the harmful effects of crude oil contaminated water on the guinea pig and the histology of its ileum.

Methodology and result: Twenty four guinea pigs were divided into four groups, comprising of control group A, and experimental groups: B, C, and D. They were allowed to acclimatize for two weeks, and were fed elephant grass and tap water. With insulin syringe, 1 mil (approximately equivalent to 10 drops) of crude oil contaminated water was administered to the experimental groups: B,C, and D; once, twice, and thrice, daily, respectively, for six weeks. It was observed that the histological architecture of group A, were intact, while that of the experimental groups were disrupted, the degree of disruption was directly proportional to the concentration of crude oil contaminated water administered.

Conclusion and application of findings: Ingestion of crude oil contaminated water is detrimental to health. The findings from this study may be useful in making valid and informed decisions for public consumption, as regards the health implication of eating and drinking crude oil contaminated food and water respectively.

Key words: Crude oil contaminated water, Guinea pig, Tap water.

INTRODUCTION

Guinea pig belongs to the kingdom: Animalia, Phylum: Chordate, Class: Mammalia, Order: Rodentia, Suborder: Hystricomorpha, Family: Caviidae, Subfamily: Caviinae, Genus: Cavia, Species: C. porcellus. (Scientific classification, the free encyclopedia). The guinea pig was most extensively implemented in research and diagnosis of infectious diseases (Reid, ME. 1958). Guinea pigs have been launched into orbital space-flight several times, first by the USSR on the Sputnik 9 biosatellite of March 9, 1961 - with a successful recovery (Gray, T. 1998). The use of guinea pigs in scientific experimentation dates back at least to the 17th century, when the Italian biologists Marcello

Malpighi and Carlo Fracassati conducted vivisections of guinea pigs in their examinations of anatomic structures (Guerrini, A. 2003). Guinea pigs played a major role in the establishment of germ theory in the late 19th century, through the experiments of Louis Pasteur, Emile Roux, and Robert Koch (Guerrini, A. 2003). In 1780, Antoine Lavoisier used a guinea pig in his experiments with the Calorimeter. (Buchholz, AC & Schoeller, DA. 2004). China also launched and recovered a biosatellite in 1990 which included guinea pigs as passengers (Timeline: China's space quest.2007). In 2004, the U.S.'s National Human Genome Research Institute announced plans to sequence

the genome of the domestic guinea pig (NHGRI, 2007). Because guinea pigs are one of the few animals which, like humans, cannot synthesize vitamin C but must obtain it from their diet, they are ideal for researching scurvy. (Gad, SC, 2007).

The word *petroleum* comes from Latin: "petra" for rock and "oleum" for oil; and also from Greek: *petra* (rock) + Latin: *oleum* (oil) (Concise Oxford English Dictionary). The term crude means the natural state, not refined (Hornby, 1998). Crude oil is a naturally occurring, flammable liquid consisting of a complex mixture of hydrocarbons of various molecular weights and other liquid organic compounds that are found in geologic formations beneath the Earth's surface (Guerriero V. *et al.* 2010 & 2011). Petroleum is a fossil fuel derived from ancient fossilized organic materials, such as zooplankton and algae. (Kvenvolden, KA, 2006). Vast quantities of these remains settled to sea or lake bottoms, mixing with sediments and being buried under anoxic conditions. (Braun, R L. & Burnham, IK, 1993). This massive organic deposit later became heated and transformed under pressure into oil. (Broad, WJ, 2010). The Nigeria Bonny light crude oil contains: Paraffins 37 % by volume, Naphthenes 54% by volume, Aromatics 9% by volume, Sulfur 0.1%, and API gravity of 36. (API, 2004)

Crude oil and refined fuel spills from tanker ship accidents have damaged natural ecosystems in Nigeria and many other places. Oil spills at sea are generally much more damaging than those on land, since they can spread for hundreds of nautical miles in a thin oil slick which can cover beaches with a thin coating of oil. This can kill sea birds, mammals, shellfish and other organisms it coats. Alterations in some haematological indices have been observed following oral gavaging of Bonny light crude oil to Guinea pigs. (Eastin W. C. Jr, & Murray, H. C. 1981; Ormseth, O. A. & Ben-David, M. 2000). Additionally, volatile low molecular weight components diffuse into the blood through the respiratory tract. After absorption via the pulmonary or gastrointestinal routes, crude oil is transported in plasma initially bound to albumin and other larger proteins to the liver (Orisakwe, O. E. *et al.*, 2004). Exposure of

Guinea pigs to crude oil caused a significant reduction in red blood cell and haematocrit values. (Daniel U O *et al.*, 2005). These results suggest that Bonny light crude oil (BLCO) caused the increased availability of crude oil hydrocarbons in the liver cells, and subsequent induction of unscheduled mitochondrion DNA synthesis, and alteration of mitochondrial/endoplasmic reticulum Ca^{2+} sequestration or Ca^{2+} concentration gradient, leading to the inhibition of Ca^{2+} influx into the cytosol. These events may explain the probable hepatotoxicity of BLCO. (Ibiba FO & Adirimo BJ, 2007). The acute toxicity of CASRN 8002-05-9 (CASRN 8002-05-9 represents all conventional crude oil), in a prenatal developmental toxicity study in rats administered CASRN 8002-05-9 via gavage, reduced maternal body weight was observed at 887 mg/kg-day. Signs of developmental toxicity consisted of reduced fetal weight, reduced fetal crown-rump length, increased numbers of resorptions and the number of dead fetuses and decreased number of live fetuses at 887 mg/kg-day. Signs of developmental toxicity consisted of increased number of resorptions, decreased litter size, decreased fetal weight, incomplete ossification of nasal bones and caudal centra and an increased incidence of pup mortality during lactation at 500 mg/kg-day. CASRN 8002-05-9 was mutagenic in bacteria *in vitro* but did not show evidence of chromosomal aberrations in mammalian cells *in vitro*. CASRN 8002-05-9 did induce chromosomal aberrations in mice *in vivo*. CASRN 8002-05-9 is irritating to rabbit skin and eyes and did not induce sensitization in guinea pigs. CASRN 8002-05-9 is carcinogenic to mice via dermal exposure. (API, 2004).

The guinea pigs were sourced from the animal house of the college of Health sciences, University of Uyo, while the crude oil (Bonny light) contaminated water was obtained from Nigerian National Petroleum Company (NNPC). The experiment was done in the research laboratory of the department of Anatomy, college of Health Sciences, University of Uyo, Nigeria.

The aim of the study is to ascertain the effect of crude oil contaminated water on the histology of

the ileum of the guinea pig. To evaluate the association between ingestion of crude oil

contaminated water and malfunctioning of the ileum.

MATERIALS AND METHODS

Healthy and robust guinea pigs, and body weight within the ranges of 200gm to 300gm, were the main criteria used in selection of guinea pigs for this study. Following acclimatization for two weeks, the guinea pigs were weighed before the commencement of experiment. They were weighed again at intervals of one week throughout the six weeks. Clean tap water was provided and left in each cage for twenty four hours throughout the six weeks for the guinea pigs to drink *ad libitum*. Elephant grass was also provided. With an insulin syringe, 1 mil (equivalent to 10 drops) of crude oil contaminated water was administered orally once, twice (morning and evening) and thrice (morning, afternoon, evening) a day to the guinea pigs in experimental groups: B, C, and D, respectively. Following exposure to chloroform, at the exact date marking the end of duration of the experiment, all the guinea pigs were sacrificed and their ileum excised, and fixed in formalin. They were subjected to a routine laboratory process. The tissue sections were examined with the aid of light microscope.

Routine laboratory process/method: the first step involved is the fixation of the ileum in 10% formal saline. The aim is to preserve the ileum in life-like manner, to prevent autolysis and putrefaction. Dehydration of the ileum immediately took place after fixation. It has to do with the removal of water from the ileum. This is made possible by introducing the ileum to different concentrations of alcohol (graded alcohol). The ileum is later cleared in a xylene. The aim of clearing is to remove alcohol from the ileum. Thereafter, the ileum

is impregnated (infiltrated) with paraffin wax in a liquid state, at a temperature slightly above its melting point (between 54 degree centigrade and 60 degree centigrade). The process of infiltration ensures that the paraffin wax replaces the xylene. It lasts for about two hours. Embedding (casting or blocking) follows suit. This has to do with the transference of the ileum from the final wax bath to a mould filled to the brim with molten wax. The ileum is inverted to free the surface to be cut from air bubbles and the ileum is orientated in such a way that the base rests on the base of the mould. Embedding is closely followed by sectioning, which involves cutting the paraffin wax embedded ileum. This is achieved by the use of a microtome. The ileum is then laid on a slide smeared with albumin as they were being cut. The ileum is subsequently floated in water bath, to flatten the ileum and remove excess grease. The next step is staining. This has to do with the dewaxing of the ileum in xylene, bringing the ileum to decreasing concentrations of alcohol, bringing the ileum to water, staining the ileum with Haematoxylin, bringing the ileum to water, bringing the ileum to acid alcohol, bringing the ileum to water, and counter staining with Eosin. The ileum was later brought to increasing concentrations of alcohol and finally brought to two variable changes of xylene. The ileum was later bloated in white filter paper. A drop of xylene was added to make it wet. It was then mounted using DPX on coverslip and allowed to dry.

Table 1: Quantity of elephant grass, water and crude oil administered to the guinea pigs.

Groups.	Elephant. Grass	Clean tap water	Crude oil contaminated water
A	not specified	20mil	(None)
B	not specified	20mil	1mil (once in the morning)
C	not specified	20mil	1mil (twice: morning & evening)
D	not specified	20mil	1mil(thrice: morning, afternoon & evening).

(1 mil is approximately equivalent to 10 drops)

RESULTS

Guinea pigs in the control group A remained healthy, robust, and gained weight throughout the experimental period. The guinea pigs in the experimental groups B, C, and D, lost appetite, hence lost weight. The observed weight loss is associated with the

concentration of crude oil administered and is also directly proportional to loss of appetite. Thus, guinea pigs in the experimental group D, administered crude oil contaminated water, thrice a day, lost weight faster than others; there was no sharp demarcation in the

quantity of weight lost between guinea pigs in experimental groups B and C; but there was between the duo and experimental group D.

Table 2: Weights in grammes, mean and the standard deviation of guinea pigs,

Groups	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42	Mean	Standard deviation
A	210	201	211	213	217	222	212	7.10
B	212	211	210	211	210	209	211	1.18
C	209	207	205	204	201	198	204	4.00
D	206	203	202	199	194	190	199	600

Based on the figures obtained, it is unlikely, that is, a likelihood of less than 5%, that the result occurred by chance. There is a real difference among groups and between groups of guinea pigs, as a consequence of different quantities of Bonny light crude oil administered. Hence, the figures obtained are statistically significant with a P- value of less than 0.05 ($P < 0.05$).

Morphological and histological results: At the end of the experiment, except the normal effects associated with tissues following routine laboratory process, the ileum become thinner, and shrunken. However, the simple columnar epithelium with its associated micro villi and crypts were intact, this is shown in plate A, the control group .Plate B, is the photomicrograph of the ileum of the guinea pigs in the experimental group B. subtle and close examination showed that the histo-

architecture of the ileum were not affected by the crude oil contaminated water. Note the perpendicularity of the simple columnar epithelium to the basement membrane; as seen in the control group A. Also note the intact mucous membrane (mucosa). The experimental group C is represented by plate C, in which the layer of epithelium along with its lamina propria had become slightly disoriented; with the columnar nature of the epithelium still discernable. In plate D, (experimental group D); the epithelium had become more disoriented, hence had lost its columnarity. The striated border(s) of the microvilli has lost its shape of finger-like projections and now assumed a brush border. The laminar propria had become much less dense; while the peyer's patch looked somewhat inflamed.

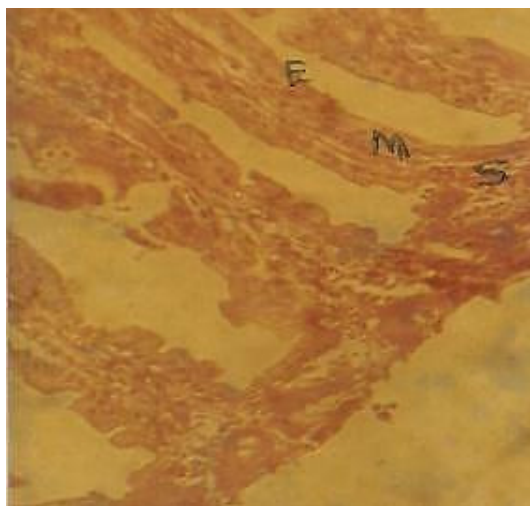


PLATE A.

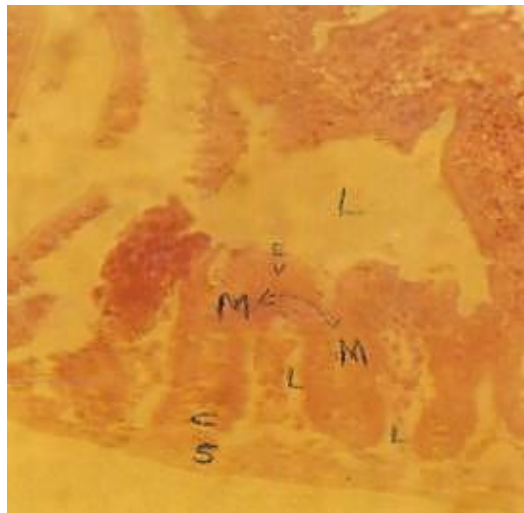


PLATE B.

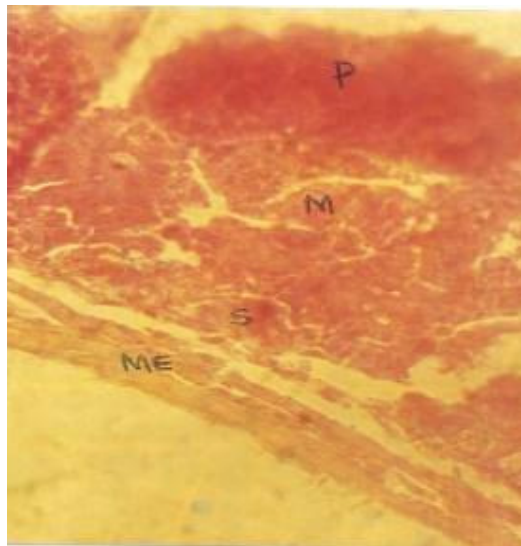
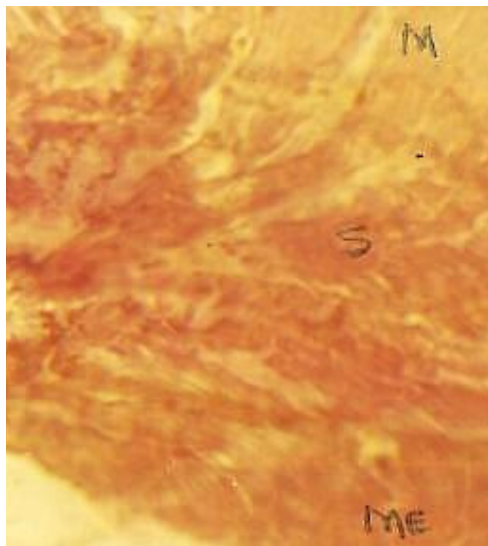


PLATE C.

PLATE D.

Legend: A = Adventitia (serosa) , C= Crypts of lieberkuhn, E = Epithelium, G = Goblet cells , L = Lumen, M = Mucosa (plicae circulars), ME = Muscularis externa, MM = Muscularis mucosa, S = Submucosa, P = Peyer's patch

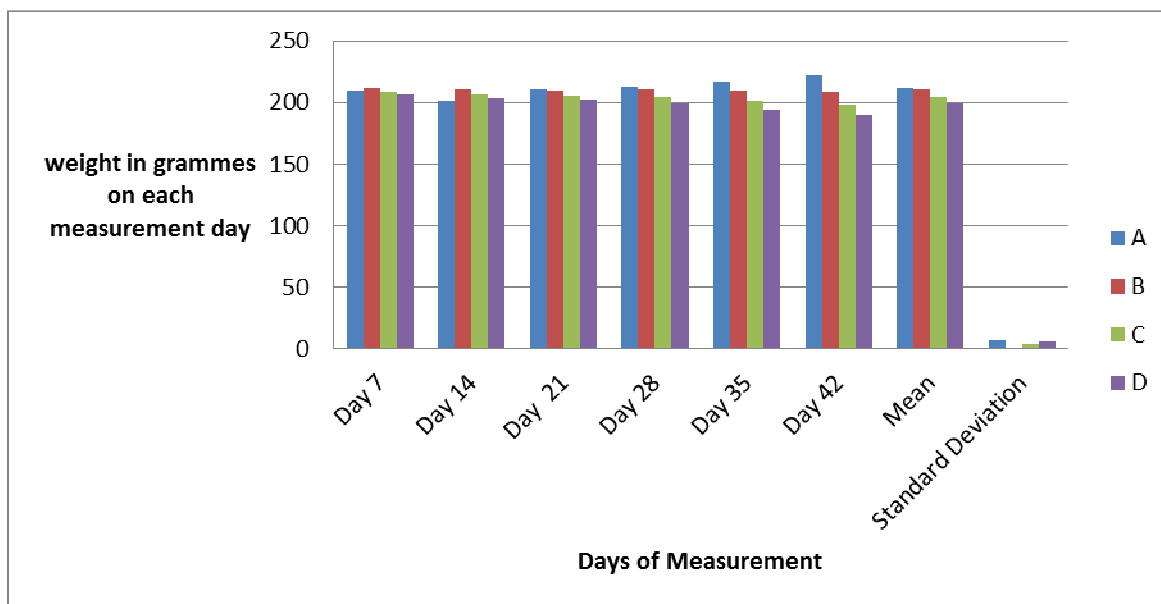


Figure 1: Graph showing the mean of the weight in grammes on each measurement day

DISCUSSION

Comparing the various plates B, C and D, with the control A; the crude oil contaminated water had little or no effect in the guinea pigs of the experimental group B; but with noted effect on those of experimental group C; as indicated in the result.. However, in the experimental group D, the effect is much more marked. The disorientation of the finger-like projections of micro villi from its normal striated border appearance to brush border-like appearance may indicate that crude oil has a coagulating effect. The crude oil caused the finger-

like projections of the microvilli to coagulate and form clusters. In this form, microvilli being reputed to be an active site of absorption, will be encumbered and hindered in the act of absorption. Hence, the malnourishment of the guinea pigs where such is seen. The observed loss of weight may be attributed to this. In an epidemiology study, blood samples were taken from 68 individuals (control n = 42) exposed to crude oil during the cleanup of a spill and the cytogenetic damage was assessed as determined by sister

chromatid exchange (SCE). Workers in the high-pressure cleaner worker category (n = 23) showed a statistically significant increase in their SCE frequency as compared to controls (Perez-Cadahia et al., 2007). In the dermal repeated-dose toxicity study, no effects on the reproductive organs were observed in male rats treated with 500 mg/kg-day (only dose tested) (Perez-

Cadahia et al., 2007). In an epidemiology study of workers exposed to crude oil, workers in the job category upstream operator offshore" had an excess risk of hematologic neoplasm (blood and bone marrow), RR 1.90, 95% CI 1.19 – 3.02 and multiple myeloma, RR 2.49, 95% CI 1.21-5.13 as compared to that of the general working population (Kirkeleit et al., 2008).

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